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Armstrong & Pilo
Canadian Patent and Trademark Agents

285 Fountain Street South
Cambridge, Ontario
Canada N3H 1J2

Telephone: 519.653.1331
Facsimile: 519.653.8453
e-mail: canpat@sentex.net
Internet: www.sentex.net/~canpat

R. Craig Armstrong
B.Eng. (Mech.), P.Eng., LL.B.

Associates:
Martin J. Marcus
B.Sc. (Hons.Chem.)

David J. Pilo
B.Sc. (Math & Phys.), LL.B.

Of Counsel:
Serge Anissimoff
B.Eng., LL.B.

June 5, 1996

Commissioner of Patents and Trademarks **BY COURIER**
Building 2, Crystal Plaza
2011 Jefferson Davis Highway
Arlington, Virginia 22202
U.S.A.

Dear Sir:

Please file the attached specification as a **new provisional patent application:**

Title:	LOW FREQUENCY MAGNETIC FIELD DESIGNED PULSES (CNps) WHICH ALTER PHYSIOLOGICAL/NEUROLOGICAL PHENOMENA
Attorney's docket no.:	UWO-25PUS
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Sole or first inventor:	**Alex W. Thomas**
Residence:	London, Ontario, Canada
Citizenship:	Canadian
Full post office address:	#1508-155 Kent Street London, Ontario Canada N6A 4V2

CAX

2-00

Second inventor: **Frank S. Prato**

Residence: London, Ontario, Canada CAX

Citizenship: Canadian

Full post office address: 408 Briarhill Avenue
London, Ontario
Canada N5Y 1P2

3-00

Third inventor: **Martin I. Kavaliers**

Residence: London, Ontario, Canada CAX

Citizenship: Canadian

Full post office address: 284 Suffolk Place
London, Ontario
Canada N6G 2A1

4-00

Fourth inventor: **Michael A. Persinger**

Residence: Sudbury, Ontario, Canada CAX

Citizenship: Canadian

Full post office address: 261 Wilson Street
Sudbury, Ontario
Canada P3E 2S3

1

Send correspondence to: R. Craig Armstrong
(Registration no. 30,910)
ARMSTRONG & PILO
285 Fountain Street South
Cambridge, Ontario
Canada N3H 1J2

Direct telephone calls to: Craig Armstrong, (519) 653-1331

We look forward to confirmation of filing in due course.

Yours very truly,

ARMSTRONG & PILO

Per:

R. Craig Armstrong
Registration No. 30,910

60/019184

United States Provisional Patent Application

Low Frequency Magnetic Field Designed Pulses (CNps) Which Alter Physiological/Neurological Phenomena

Inventors:

Mr. Alex W Thomas

Dr. Frank S Prato

Dr. Martin Kavaliers

Dr. Michael A Persinger

Filed June 6, 1996

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98/019184

1 Patent Title

Low Frequency Magnetic Field Designed Pulses (CNps) Which Alter Physiological/Neurological Phenomena

2 Inventors

Mr. Alex W Thomas, BA, MSc Graduate Student,
Neuroscience Programme, University of Western Ontario,
#1508-155 Kent Street, London, Ontario, CANADA, N6A 4V2
ph. 519-645-0844
fax 519-645-0846
email athomas@lri.stjosephs.london.on.ca

Dr. Frank S Prato, PhD, FCCPM, ABMP,
Professor, Department of Diagnostic Radiology & Nuclear Medicine, University of Western Ontario
Chairman, Imaging Division, Lawson Research Institute
268 Grosvenor Street, London, Ontario, CANADA, N6A 4V2
ph. 519-646-6100 x4140
fax 519-646-6135
email prato@lri.stjosephs.london.on.ca

and

408 Briarhill Avenue, London, Ontario, CANADA, N5Y 1P2
ph. 519-659-7023

Dr. Martin Kavaliers, PhD, Professor,
Division of Oral Biology, Faculty of Dentistry, University of Western Ontario London, Ontario, CANADA
ph. 519-661-3327
fax 519-991-3675
email kavalier@julian.uw.ca

Dr. Michael A Persinger, PhD, Professor,
Department of Psychology, Laurentian University Sudbury, Ontario, CANADA, P3E 2C6
ph. 705-675-4824

- Note all inventors are Canadian and have an equal, i.e. 25%, share.

3 Background of the Invention

Although endogenous magnetic field waveforms exist in plants and animals, the direct control and alteration of the waveforms using non-invasive technology has not, to date, been effective. We propose a design method by which an exogenous magnetic field waveform can be externally or internally applied such that the underlying physiological/neurological processes can be altered. This design is based on knowledge regarding the underlying physiological/neurological process which is to be altered. Such design is believed to be sufficiently specific such that only targeted processes will be affected.

4 Brief Description of the Drawing

Fig. 1a shows an example of a CNps pulse. This pulse, designed and implemented by Mr. AW Thomas and hence called the "Thomas Pulse", has been used to alter the response of the opioid system. It shows the three unique characteristics of the CNps pulse:

a) the refractory period
b) the decreasing (or increasing) frequency
c) the fast rise times

Fig.1b is an enlargement on the time scale of one segment of this CNps showing in greater detail the nature of the fast rise times.

Fig.2 shows the effect of exposure of snails to a CNps which in this case was the Thomas Pulse. Analgaesic or anti-nociception levels in the land snail, *Cepaea nemoralis*, was increased as compared to sham controls when the snails were exposed for 15 minutes to the CNps. When the snails were also injected with μ and δ opioid antagonists, some of the analgaesic effect of the CNps was attenuated. When a κ antagonist was given along with the CNps, there was no apparent effect of the antagonist on the levels of nociception.

Fig.3 shows the results of experiments in which the increase in analgaesia produced by the CNps (see Fig.2) was studied with respect to tolerance. That is, since at least some of the effect is believed to be related to the opioid system, then the question of whether or not the land snail developed a form of classical opioid tolerance to repeat CNps exposures was investigated. Fig.3a shows that tolerance does occur and the effectiveness of a 15 minute magnetic field exposure decreased with daily repeated exposures. The greatest decrease in effectiveness occurred within the first 4 days. Fig.3b shows how the analgaesia decreases with time after a 15 minute exposure to the CNps for the first day of treatment. Fig.3c is a "3D plot" which shows the effects of CNps exposed snails as in Fig.3a and Fig.3b but for all the 10 exposure days. Fig.3d shows the "3D plot" of the data for the sham CNps exposed snails.

Fig.4 shows that the CNps can also increase opioid-induced analgaesia.

5 Detailed Description

5.1 Background

Conventional wisdom asserts that magnetic fields arise as an insignificant byproduct of brain electrical activity. Further, that the application of an external weak magnetic field, as proposed here, could not modify the brain electrical activity because to do so would require the induction of currents above a certain threshold and this is not possible given the conductivity and background electrical noise in the brain. However, this bias is not consistent with the biological literature on effects of weak non-ionising non-thermal low frequency magnetic fields. Starting with the well established, now uncontested, evidence that animals can use earth strength magnetic fields for orientation and migration [e.g. Mather & Baker 1980; Phillips & Borland 1992; Phillips & Sayeed 1993] to effects on neurochemical and cellular actions [e.g. Frey 1994] there is extensive evidence that magnetic fields can have significant biological effects. In fact, it has been known for more than the last 10 years that a very weak magnetic field (comparable in strength to that of the earth) can induce alterations in the electrical activity of neurons as measured using micro-electrodes [e.g. Semm *et al* 1980, Semm & Beason 1990; Lohmann & Willows 1991]. Given this background, it is quite feasible that the magnetic pulses [CNps] we are describing can be sensed. Therefore, the present proposal is consistent with the literature if not with the opinions of some conservative scientists.

5.2 Pulse Design

5.2.1 Background

For more than 20 years, EEG evidence has shown that the frequency of electrical activity in the brain varies between 0 and approximately 70Hz. What is of controversy now is the importance of frequency and phase synchronisation for behavioural and cognitive functions. One current theory is that this synchronisation is essential for brain function, allowing collections of neurons distributed in small local regions of the brain and those distributed diffusely throughout larger regions of the brain to act together in the deposition and retrieval of information. Based on the current literature, there is a suggestion that the application of simple sinusoidal magnetic fields can disrupt or alter this synchronisation or even the underlying frequencies [Lyskov 1993; Bell *et al* 1992a,b,1994]. For example, the intensity and severity of experimentally induced epilepsy model in rats can be reduced by exposure to sinusoidal magnetic fields [Ossenkopp & Cain 1988]. Although controversial, there is some evidence that this also has been seen in humans with a history of epilepsy [Fuller *et al* 1995]. Another example is the treatment of depression. It is possible that patients with depression have an abnormal frequency synchronisation in the brain which inhibits normal brain function [Zyss 1994]. This can be disrupted with electro-convulsive shock therapy (ECT). More recently, it has been shown that the application of strong external magnetic fields can also have a positive effect in the relief of depression [Fleischmann *et al* 1995; Holden 1995]; properly designed CNps should be tried next. Note that it is well-accepted that the existing brain frequency and synchronicity can be altered or "locked" to an external physical stimulus. e.g. In epileptics, a flashing light at 3Hz can cause global synchronisation and a *grand mal* seizure; in other individuals with partial epilepsy signs, such exposure often causes discomfort.

What is being debated is whether or not magnetic fields can affect the synchronicity and frequency of endogenous brain electrical activity. There is mounting evidence that weak magnetic fields can affect brain function [Bell *et al* 1992a,b,1994]. We and others have shown that weak magnetic fields can affect the opioid system functions associated with pain sensitivity in invertebrates (molluscs) and vertebrates (birds, rodents and indirectly humans) [Betancur *et al* 1994; Del Seppia *et al* 1995; Kavaliers & Ossenkopp 1985, 1986a-c, 1988; Kavaliers *et al* 1994; Ossenkopp & Kavaliers 1987; Papi *et al* 1992; Prato *et al* 1995]. We have shown that simple sinusoidal fields can attenuate opioid-induced analgaesia and, more recently, that they can increase opioid-induced pain-inhibition or analgaesia and even induce analgaesia. However, like the treatment of epilepsy, the effects have been relatively small. What we are proposing is that these effects can be increased by designing CNps which will be more effective in modifying frequency or disrupting synchronisation.

5.2.2 Design Parameters

The design philosophy is that to disrupt a brain oscillation frequency f, and specifically to lower it, it is best to expose the brain, or the appropriate part thereof, to a frequency $f' > f$ and then to sweep the frequency down to 0Hz. This will ensure that at one point in time, the external applied frequency and the internal frequency are matched, i.e. in resonance. It has been found that this approach increases the chances of modifying the internal frequency by a process of entrainment. Typically, the frequency sweep is applied for approximately 2sec and then for approximately 2.5sec, no external fields are applied. During this "quiet time", the brain recovers at a new frequency f" which is usually lower than f'. Then the external field is applied again. Note that a simple sine-wave at a lower frequency ($f' < f$) might have a small effect but entrainment and disruption of the endogenous oscillators is more effective if the frequencies sweep from higher to lower values and there is a refractory period. We further believe that the pulse will be more effective if the time-varying magnetic field has fast rise- and fall-times to increase the probability of induced currents. To achieve this, high-frequency "spikes" are added to the fundamental carrier frequency which is being swept from high to low frequencies. In effect, these high-frequency spikes induce current and the low fundamental frequency determines the period between the induced currents. An analogy would be the slowing down of a swing by applying sharp thrusts at a frequency just below the fundamental frequency of the swing. Of course, the thrusts to the swing will be most effective if they are applied at the optimal point in the phase of the swing. Thus synchronisation is needed between both frequency and phase.

In summary, we are talking about a general design concept. The design has the following unique attributes (see Fig.1): 1) frequency sweep from high ($f' > f$) to low values ($f' < f$); 2) a quiescent period; 3) sharp rise- and fall-times on top of the lower "fundamental" frequency. Note that this is the waveform used to reduce the endogenous frequencies. In principle, endogenous frequencies could be increased by application of the CNps pulse with an increasing frequency rather than a decreasing fundamental frequency. We consider this alternate form of the CNps to be part of this design for patent purposes.

5.3 Applications

5.3.1 Enhancement of Opioid-induced Analgaesia and Induction of Analgaesia

We now have extensive proof in land snails that one such CNps designed to be effective in disrupting frequencies between 10 and 20Hz is effective in both increasing opioid-induced analgaesia (see Fig.4) and inducing analgaesia (see Figs.2&3) [Thomas *et al* 1996a,b]. We have shown that a smaller effect is possible using just a single frequency sinusoidal magnetic field waveform [Prato *et al* 1996a,b; Kavaliers *et al* 1996]. But, the effect, as we predicted, is greater using the CNps pulse which synchronises phase and frequency, applies "induced current" fast-switched pulses and allows for a refractory period. Within the next several months, we will accumulate data in rodents to establish the effectiveness of this approach in mammalian systems. Results of our prior investigations have established that the effects of magnetic fields on molluscs and mammals are equivalent [Kavaliers *et al* 1994].

There is some effect of tolerance (see Fig.3), as there should be if we are continually stimulating the opioid system. Experiments are needed to determine the frequency of application of the CNps for maximum effect. e.g. should we continually expose for a minute followed by a 10min rest followed by another 1min exposure or should the exposure be continuous for 10min and then have a 10min break, etc. Patterns of tolerance are dependent on the components of the opioid systems that are stimulated. We need to delineate the exact components that are being activated. The magnetic fields may function as an adjunct to decrease tolerance in cases of chronic opioid administration [Kavaliers & Ossenkopp 1985].

5.3.2 Disruption of the Human Vestibular System

We have also recently designed a pulse to interfere with the human vestibular (balance) system. The oscillatory frequency for the human vestibular system is approximately 100Hz, therefore we increased the frequency of the CNps pulse which we used to induce analgaesia by a factor of 3 (the present limit of our exposure system). To date, we have exposed 4 human volunteers and have effectively reduced "balance" in 3 of them. We will now apply to the Human Experimentation Committee at the UWO to evaluate the effectiveness of this pulse using non-laboratory human volunteers. This will demonstrate the general use of this concept of pulse design.

6 Summary of Applications of the CNps Pulse

6.1 Applications Supported by Direct Data from our Laboratory Using CNps

- 6.1.1 Increased opioid-induced analgaesia (seen in snails; see Fig.4) [Thomas 1996a,b]
- 6.1.2 Induced analgaesia (seen in snails; see Figs.2&3) [Thomas 1996a,b]
- 6.1.3 Altered vestibular function/balance (seen in humans)

6.2 Applications Inferred by Direct Data from our Laboratory Using Single Sinusoidal Pulses

- 6.2.1 Decreased opioid-induced analgaesia (seen in snails and mice) [Kavaliers *et al* 1994]
- 6.2.2 Decreased intensity and severity of epileptic-like seizures (seen in rats, also in humans by other investigators) [Ossenkopp & Cain 1988]
- 6.2.3 Decreased tolerance to opiates in rodents [Kavaliers & Ossenkopp 1985]
- 6.2.4 Improvements in rates of learning [Kavaliers *et al* 1996]

6.3 Applications Inferred by Direct Data from our Laboratory Using Complicated Pulses but not CNps

- 6.3.1 Decreased blood-brain barrier permeability (seen in rats exposed to strong DC fields and summed sinusoidal fields) [Prato *et al* 1990,1994; Shivers *et al* 1986]
- 6.3.2 Increased tooth dentin formation (seen in mice exposed to magnetic fields from MRI) [Kwong-Hing *et al* 1989]
- 6.3.3 Increased and decreased second messenger activity in transformed human blood cells (seen in transformed human cells of HL60 and Jurkat lines) [Carson *et al* 1990]

6.4 Applications Inferred by Results Reported Using Other Pulse Sequences Where We Propose that the Effect Will be Increased by Using the CNps

- 6.4.1 Reduced severity of epileptic episodes in patients with epilepsy
- 6.4.2 Induced mild epileptic Episode in patients with epilepsy [Fuller *et al* 1995]
- 6.4.3 Altered second messenger activity in human blood cells [Walleczek & Liburdy 1990; Walleczek 1992; Lindstrom *et al* 1993]
- 6.4.4 Altered visual perception and visual short-term memory [Beckers & Homberg 1991]
- 6.4.5 Modified brain performance such as reaction time [Lyskov *et al* 1993]
- 6.4.6 Treatment of depression in humans [Holden 1995; Fleischmann *et al* 1995]
- 6.4.7 Treatment of schizophrenia in humans [Grisaru *et al* 1994]
- 6.4.8 Modified (and controlled) hormone secretion, e.g. Melatonin [Reiter & Richardson 1992; Brahim & Touitou 1995; Selmaoui & Touitou 1995]
- 6.4.9 Modified bone growth [Bassett *et al* 1981]
- 6.4.10 Treatment of soft tissue injury [Canady & Lee 1991; Ito & Bassett 1983; Sisken *et al* 1990]
- 6.4.11 Treatment of patients with Parkinson's Disease [Pascual-Leone *et al* 1994]
- 6.4.12 Treatment of patients with AIDS
- 6.4.13 Treatment of patients with cancer

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exogenously altering the electromagnetic fields can have profound effects on the land snail. Snails treated with SCH 34826 (specific enkephalinase inhibitor) were shown to have increased antinociception, and using a continuous 60 Hz magnetic field, the antinociception was significantly attenuated. The magnetic field design [Prato *et al* 1995] was based on the theory of parametric resonance involving the ions potassium and calcium. Further investigation prompted by the early results of CNps treatments resulted in the enhancement of antinociception in SCH treated snails at 30 and 60 Hz based on magnetic field design parameters predicted by Prato's calculations. These findings have been repeated in blind studies and have been the first experiments to elicit an exact behavioral response of antinociception using simple magnetic fields as predicted by a mathematical model of the interaction of ion and magnetic field parameters.

Although of immense theoretical importance, the effect of the simple magnetic field design was weak, explaining approximately 10% of the variance in statistical analysis. However, CNps treatment of non-SCH treated snails resulted in explaining approximately 77% of the variance between CNps treated snails and those receiving sham CNps treatments of 15 minute duration.

It is believed that one of the effects of opioids is on opioid receptor mediated intracellular potassium and calcium concentration. By increasing opioid receptor activation, the intracellular potassium level is increased and an extended refractory period is achieved. With an extended refractory period, less signaling neurotransmitter is released at the synapse and messaging then slows, resulting in a behavioral response of lessened nociception, or antinociception.

Selected opiate receptor antagonists used in this study:

Antagonist	Dosage	Preparation	Target
Naloxone (Nx)	1.0 $\mu g/\mu l$	saline, immed. inj.	gen. μ, δ
Naloxazine (Nz)	1.0 $\mu g/\mu l$	Etoh, 24 hr pre inj.	μ_1
Naltrindole (Nt)	0.1 $\mu g/\mu l$	saline, immed. inj.	δ_2, gen δ
β-funaltrexamine (B-fna)	1.0 $\mu g/\mu l$	saline, 24 hr pre inj.	gen μ, μ_1
nor-binaltorphamine (Nb)	1.0 $\mu g/\mu l$	saline, 24 hr pre inj.	κ
ICI-174864 (ICI)	1.0 $\mu g/\mu l$	saline, immed. inj.	gen δ

All antagonists and vehicles were delivered via 1.0 μl injection.

Snails were randomly selected from the colony terrariums and hydrated for approximately 30 minutes in a partially covered translucent container (one gallon pail). After hydration, 12 subjects were selected from the active hydrated population and placed in a covered translucent exposure container (Canlab weight dish, 250 ml) . Subjects are then pretested for nociception latency on a hot-water controlled hotplate device confirmed at 40±0.2°C. Subjects were then injected with one microlitre of one of the selected opiate antagonists, vehicles, or not injected, and immediately returned to the container and then exposed as a group to either a sham (3-d zeroed earth magnetic field) or active CNps field (3-d zeroed field with an earth strength CNps field on the z axis) for a period of 15 minutes. Immediately after the exposure, subjects are retested for nociceptive latency and then returned to their home cages. Some selected antagonists and their vehicle solution required preinjection 24 hours before testing. These subjects were injected and housed in perforated lid containers in the home area until the day of testing.

All sham exposure groups were combined, as there were no significant differences. All groups that were CNps exposed for 15 minutes, Con (no injection), Etoh (ethanol detergent vehicle), Sal (saline vehicle), B-fna (β-funaltrexamine), Nz (naloxazine), Nx (naloxone), Nt (naltrindole), ICI (ICI-174864) and Nb (nor-binaltorphimine) recorded significantly higher latencies than the Shams ($DF[1,213]$, $F=670$, $P<.0001$, $Eta^2=.76$). There were no significant differences among the antagonists, with the exception of the κ receptor antagonist nor-binaltorphimine (Tukey's HSD). The three control groups were significantly higher than the antagonist groups with the exception of nor-binaltorphimine (Tukey's HSD). Error bars represent SE of Mean.

8.3 Figure 3: Figures a, b, c and d of CNps tolerance study

3a) CNps tolerance at 15 min. post exposure
3b) CNps induced antinociception half-life
3c) CNps exposed - tested daily (sham exposed day 10)
3d) CNps sham exposure - tested daily (exposed to CNps day 10)

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8 Appendices

8.1 Figures 1a and 1b

The Thomas Pulse is one example of a CNps. This pulse was designed to alter opioid function in the land snail, *Cepaea nemoralis*. This diagram shows the three unique characteristics of the pulse: monotonically decreasing frequency, fast rise times or spikes and a refractory period. Fig.1b is a representation of a portion of the CNps in an expanded time scale.

8.2 Figure 2: Effect of exposure to CNps and opiate antagonists

As has been shown in previous research by Prato and Kavaliers [Prato *et al* 1995; Kavaliers *et al* 1994],

The development of tolerance (lessened dose dependent effect over time) is a well known phenomena associated with the habitual acute-dose exposure to opiate compounds. If, has been postulated, the antinociceptive effect of CNps exposure is wholly or partially mediated by endogenous opioid systems, then repeated acute exposures to CNps should produce a classical tolerance profile within the land snail (*Cepaea nemoralis*).

Individually numbered groups of 15 snails (eight groups) were exposed to either a sham or CNps condition for 15 or 30 minutes each day for nine consecutive days, or only on days one and nine. On the tenth day of testing, the sham and CNps exposure conditions were reveresed for groups that had been consecutively exposed for nine days to provide an assessment of any confounding training effect.

Immediately before and after exposure and at 15, 30 and 60 minutes post-exposure, each snail was individually tested for aversive response latency (anterior footpad lift) on a hot water controlled hotplate with a polished stainless steel surface (texturally acceptable to the snail) set to 40±0.2°C. There were no significant deviations within the pre-exposure latencies recorded. The pre-exposure latencies were covaried to the post-exposure latencies to remove any bias of individual variability.

As may be clearly seen in the following ancova table, the effect of CNps exposure versus the sham exposure produced an effect that explains approximately 91% of the variance in the model.

Figure 3a details the aversive response latency as recorded at 15 minutes post exposure from day one to day nine in the CNps and sham exposure conditions.

Figure 3b details the antinociception half-life as recorded in the response latency from the pre-exposure to 60 minutes post exposure on day one for the CNps and sham exposure conditions. It may be noted that at 60 minutes post exposure there is still a significantly increased latency in CNps exposed snails versus sham exposed.

Figure 3c (3D rendering of tolerance and antinociception half-life) details the aversive response *Latency* in seconds after exposure to the CNps conditions (15 and 30 minute exposures combined, no significant differences after day three). It may be noted that the day 10 exposure to the sham condition produced a drop in latency to sham exposure levels consistent with naive snails. The formation of a classic tolerance-type response may be noted with asymptote at approximately day four. However, day nine still shows a significant degree of antinociception compared to sham groups (Tukey's HSD).

Figure 3d (3D rendering of tolerance and antinociception half-life) details the aversive response *Latency* in seconds after exposure to a sham condition (15 and 30 minute exposures were combined, no significant differences). Daily fluctuations were not significant, although a significant increase is shown on day ten when exposure conditions were reversed. The latencies recorded on day 10 (CNps exposed after 9 days of sham exposure and repeated testing) were equivalent to naive snails; hence it may be supposed that training effects did not play a role in the response latencies of CNps exposed snails.

The following ancova table (SPSS) highlights the main effects and interactions:

Tests of Between-Subjects Effects.

Tests of Significance for T1 using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN+RESIDUAL	213.07	108	1.97		
REGRESSION	13.89	2	6.95	3.52	.033
MAG	2250.60	1	2250.60	1140.76	.000
TIME	6.05	1	6.05	3.07	.083
TEST	12.60	1	12.60	6.39	.013
MAG BY TIME	23.08	1	23.08	11.70	.001
MAG BY TEST	10.16	1	10.16	5.15	.025
TIME BY TEST	16.14	1	16.14	8.18	.005
MAG BY TIME BY TEST	.03	1	.03	.01	.904

- -

Effect Size Measures

	Partial

Source of Variation	ETA Sqd	
Regression	.061	(degree of covariance with pre-exp)
MAG	.914	(effect of exposure to CNps vs. sham)
TIME	.028	(effect of 15 vs. 30 minute exposure)
TEST	.056	(effect of tolerance)
MAG BY TIME	.098	(interaction of CNps and length of exp)
MAG BY TEST	.046	(interaction of CNps and tolerance)
TIME BY TEST	.070	(interaction of len of exp and tol)
MAG BY TIME BY TEST	.000	(not significant)

8.4

Figure 4 shows evidence for the ability of the CNps to increase opioid-induced analgaesia. When snails were given the enkephalinase inhibitor, SCH34826, latency levels increased from basal values of 4-5sec (see sham no injection) to approximately 12sec (see sham, SCH). However, if they were exposed to the CNps known as the Thomas Pulse at the same time, the latency levels increased to 16sec (see CNps, SCH). Also, this figure shows the effect of a saline injection (middle panel) and another example of the induction of analgaesia (middle and right panel) caused by exposure to the CNps alone. Similar results are shown in Fig.3.

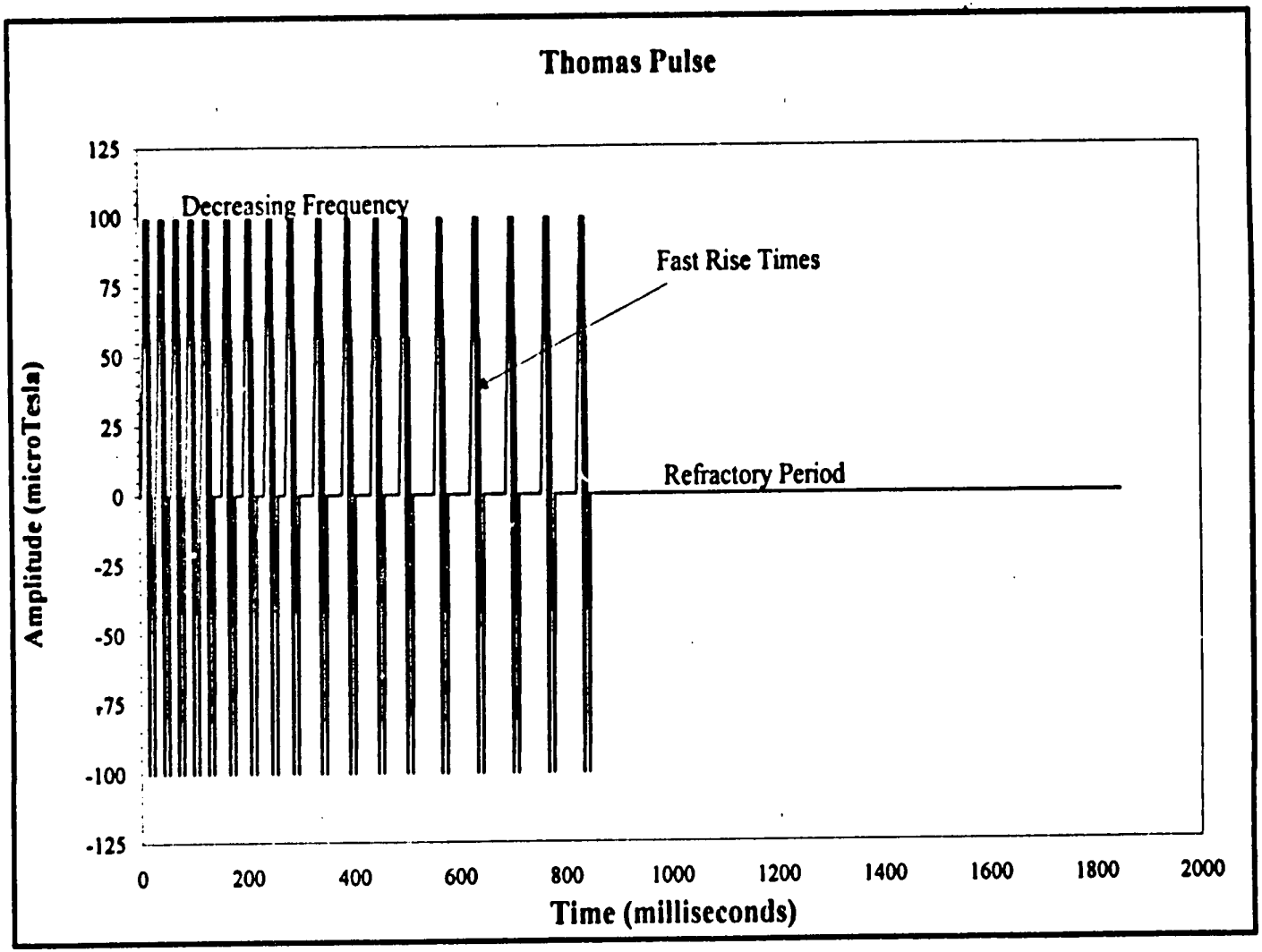

Fig 1a)

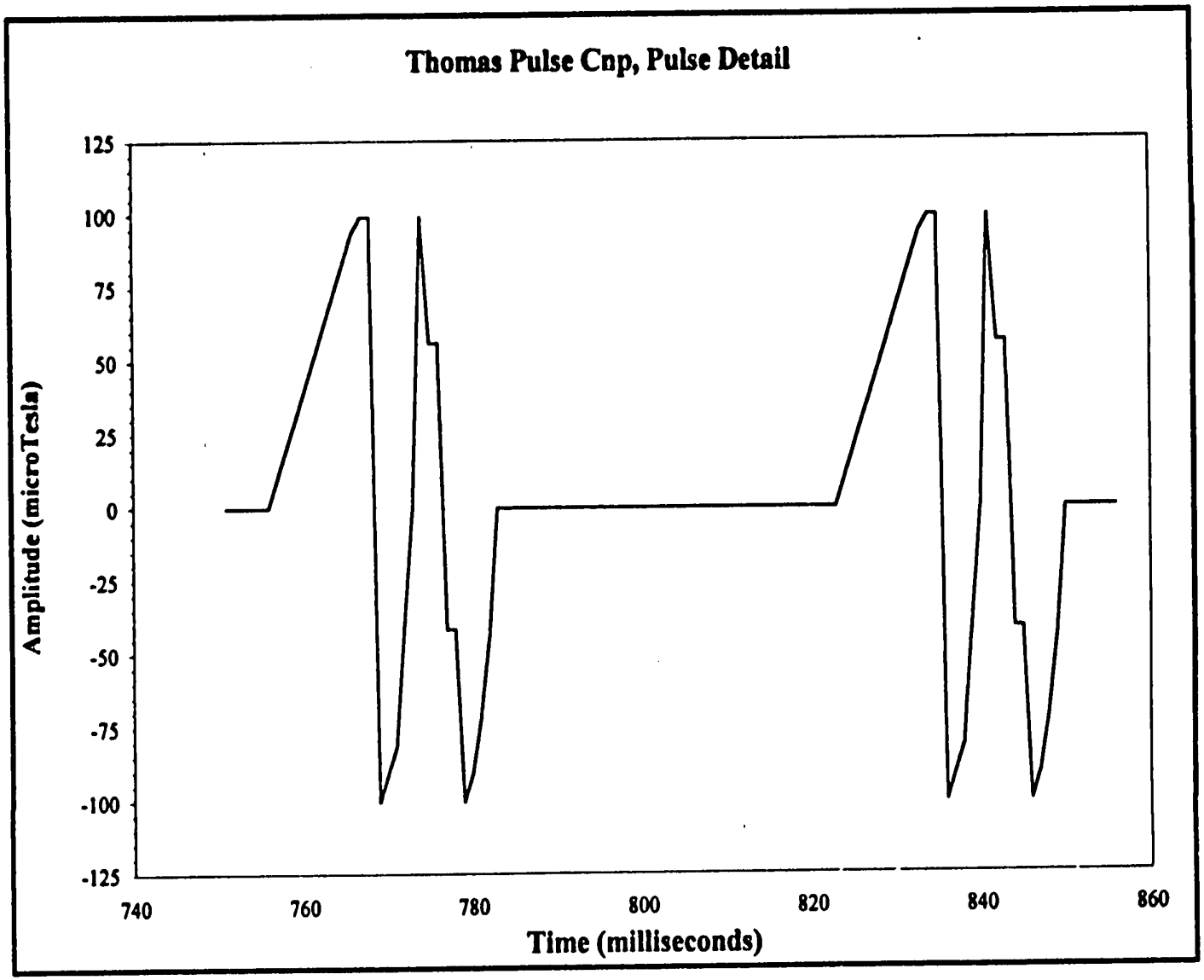

Fig 1b)

FIGURE THREE of CNp Tolerance Study

Cnp Tolerance at 15 Min. post exposure

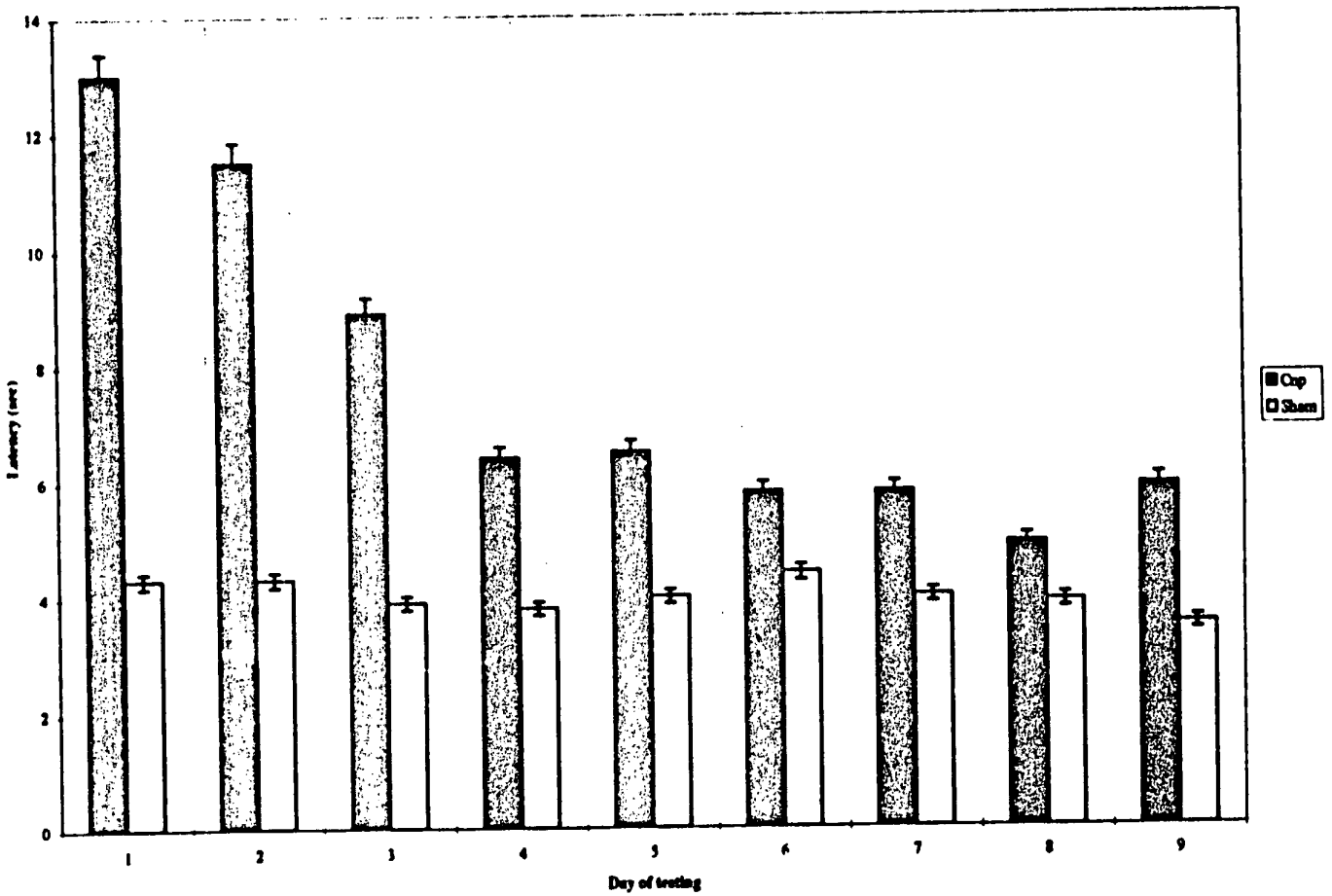

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Fig 3a)

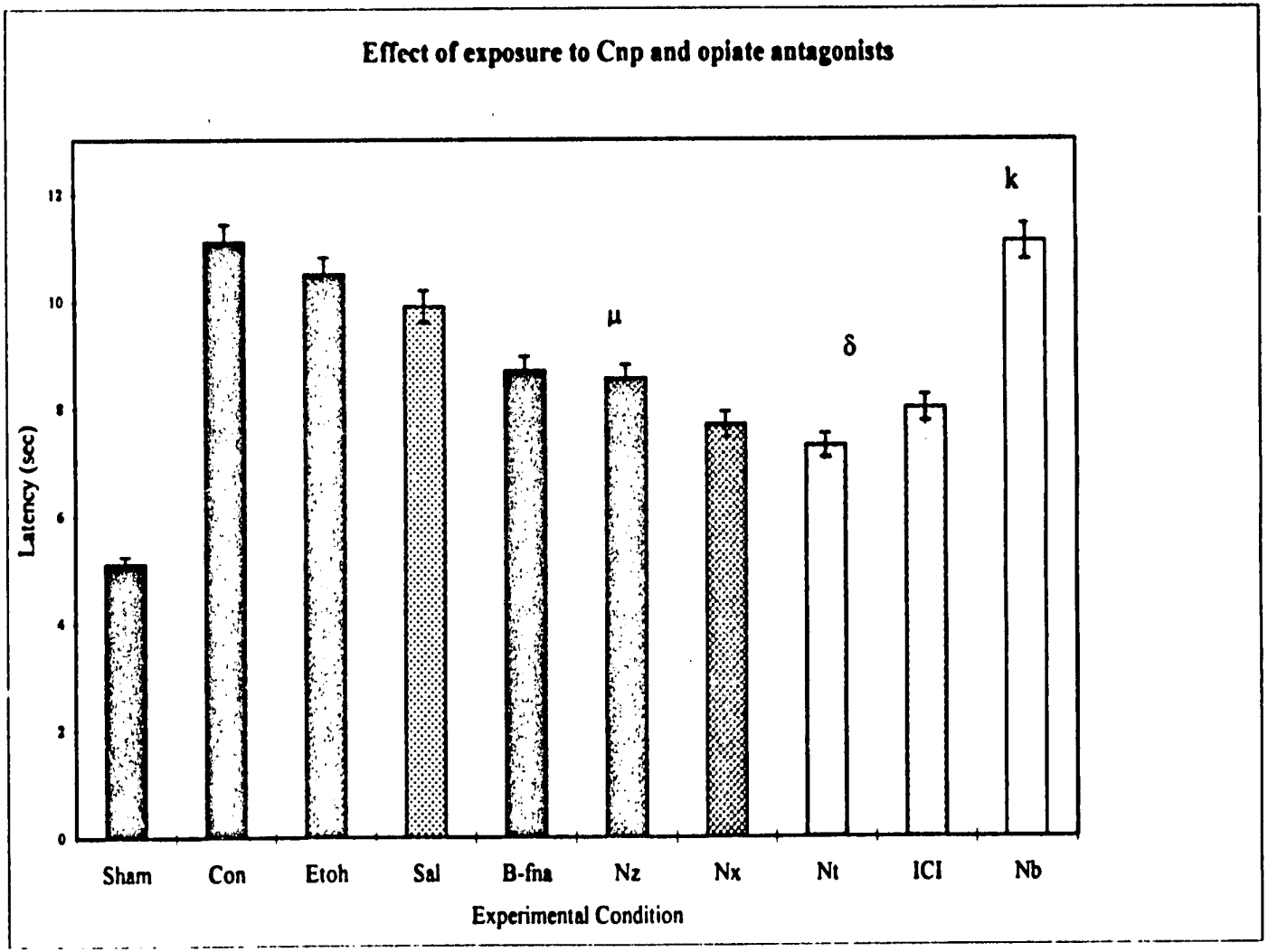

Fig 2

FIGURE FOUR of CNp Tolerance Study

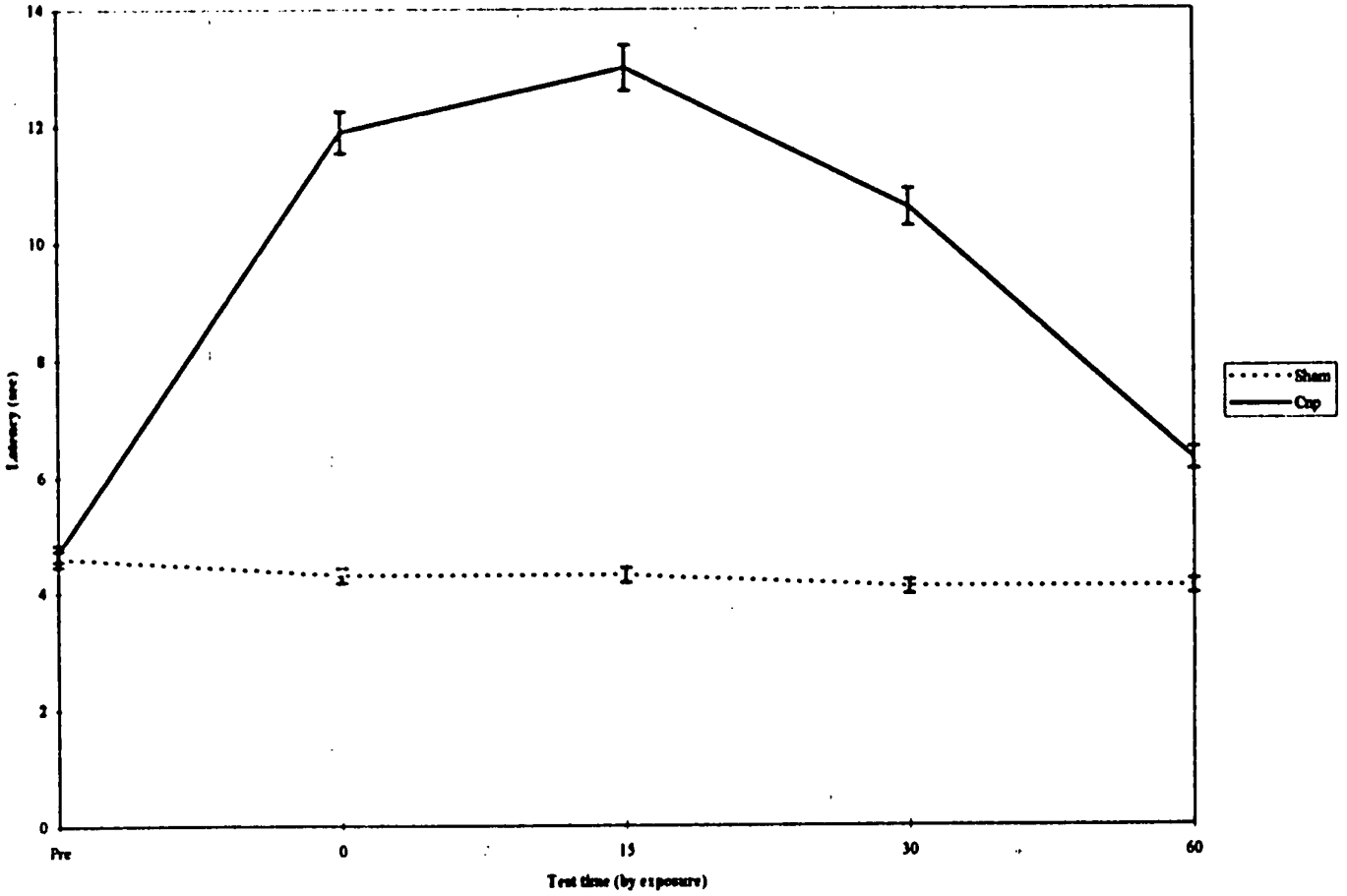

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Fig 3b)

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FIGURE ONE of CNp Tolerance Study

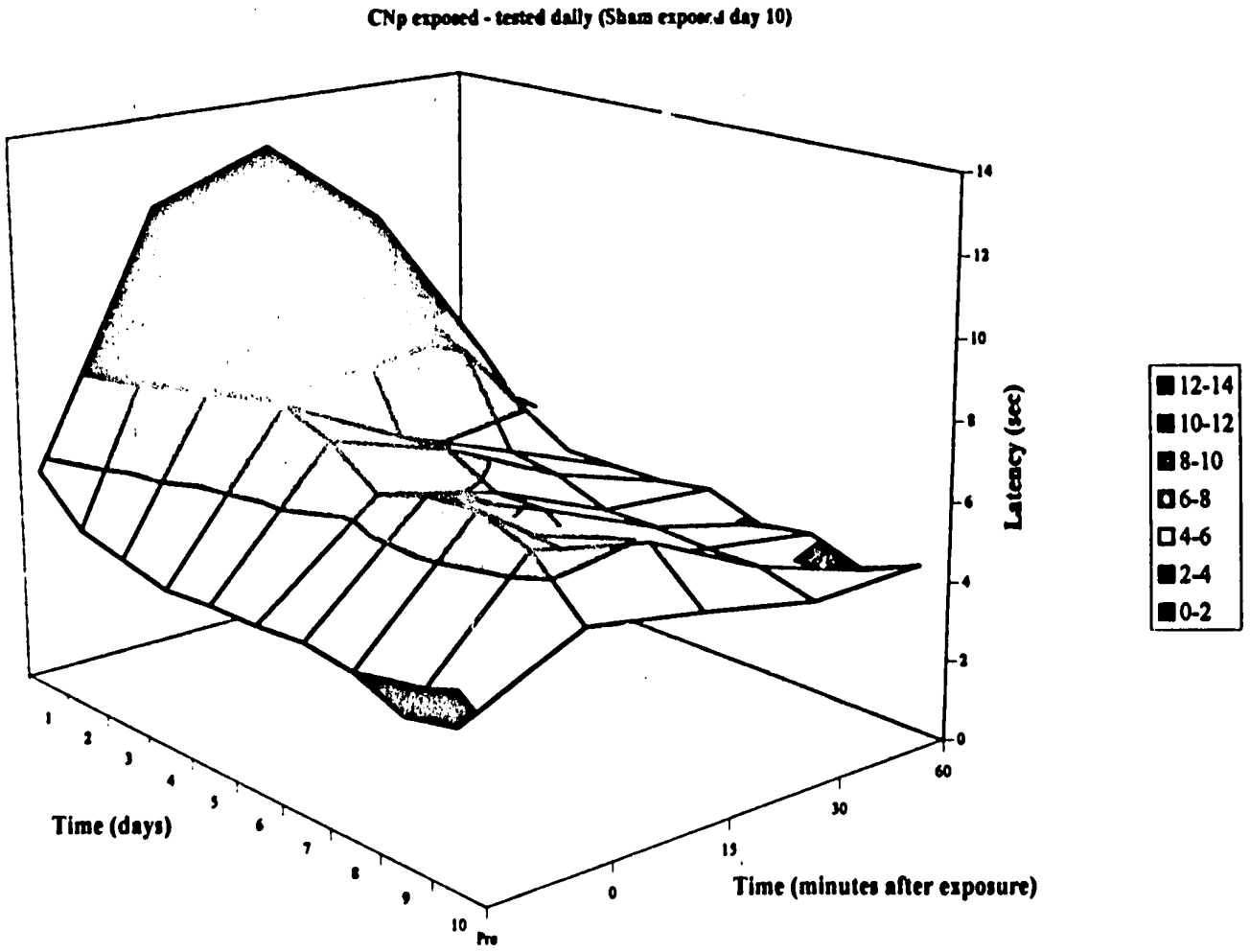

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Fig 3c)

FIGURE TWO of CNp Tolerance Study

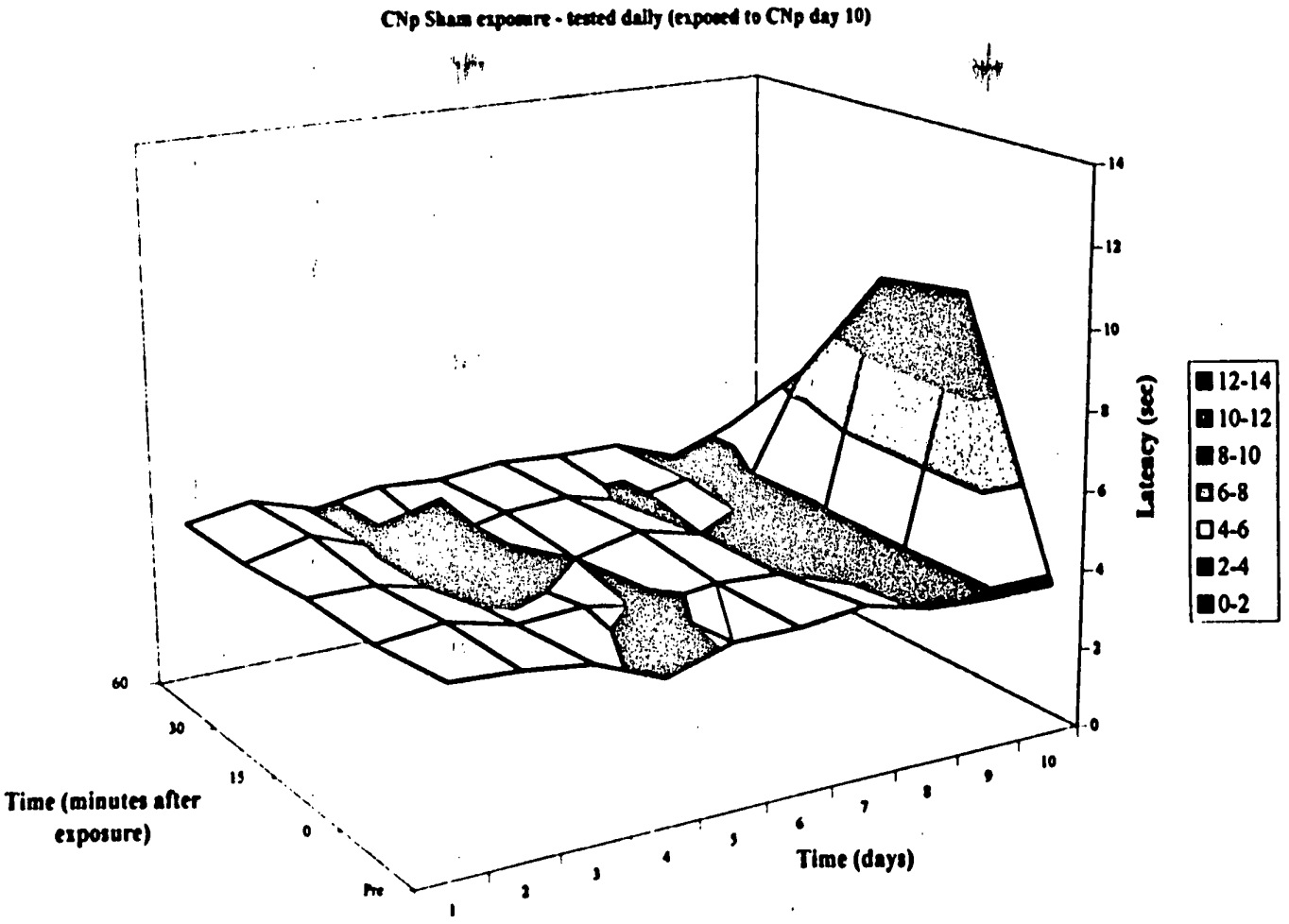

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Fig 3d)

Part of the Data on the Evaluation of the CNp (Thomas pulse)

Effect of CNp (Thomas pulse) on *Cepaea nemoralis*

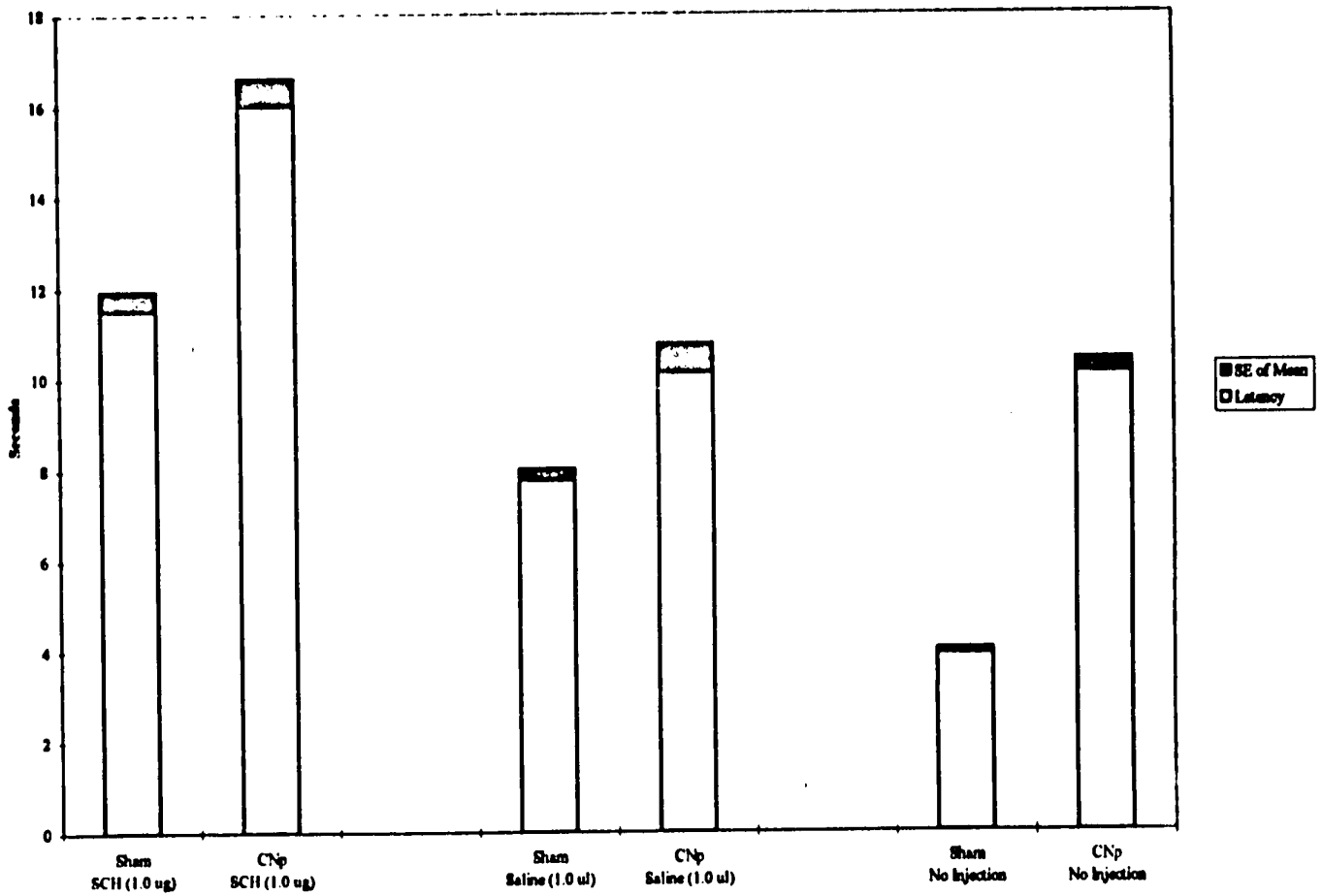

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Fig 4